



1

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Contents

- Why did we take the challenge?
- Implementation in the Laboratory and Accreditation
- Use of MPS in Missing Persons and History Projects
- Final remarks



2

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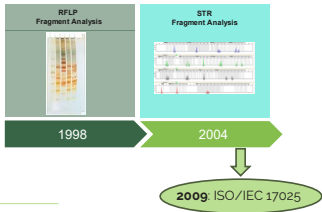
The laboratory

- Clinical and forensic genetics since 1998
- Accredited (UNE-EN ISO/IEC 17025) in the forensic genetics area
- Recognized by the Spanish Ministry of Justice
- Collaborating with official institutions
- Staff: 2 Forensic Specialists, 2 Laboratory Technicians and 1 Forensic Lab Lead
- Annually receiving ~900 requests for diverse kinship analysis and ~200 bone samples for MP studies
- Processing ~3000 DNA samples/ year



3

Technology evolution in the lab



4

4

Challenges to face in the recent past

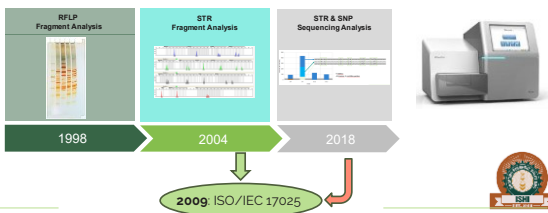
- Huge increase in service requests
- Old CE genetic analyzer and need to acquire a new software version with several licences
- High pressure to get shorter TATs
- Need to use more than one kit per sample to get enough markers typed
- Increase of the number of high challenging samples



5

5

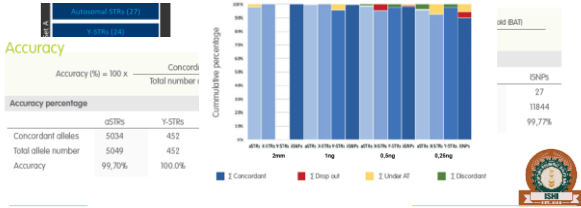
Technology evolution in the laboratory



6

6

ForenSeq® Signature Prep Kit Validation studies



ForenSeq® Signature Prep Kit Validation studies

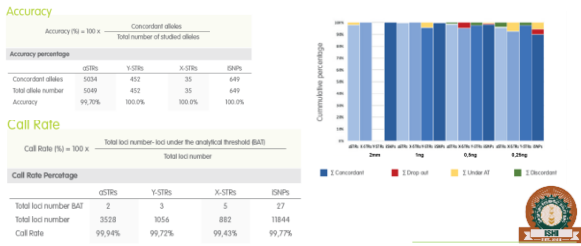


Table 1. Statistic comparison between obtained W value in samples typed by CE vs MPS

Sample number	W value (CE)	W value (MPS)
0101	0.00	0.00
0102	0.00	0.00
0103	0.00	0.00
0104	0.00	0.00
0105	0.00	0.00
0106	0.00	0.00
0107	0.00	0.00

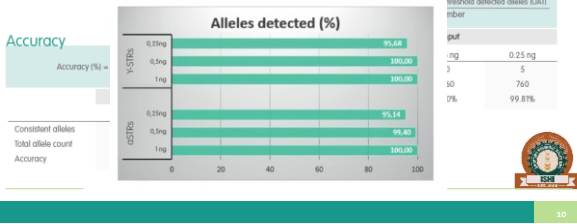
Increasing the discrimination power of ancestry- and identity informative SNP loci within the ForenSeq® DNA Signature Prep Kit

Improved pairwise kinship analysis using massively parallel sequencing

Next-generation sequencing (NGS) is revolutionizing forensic DNA analysis by providing high-resolution genotyping across the genome.

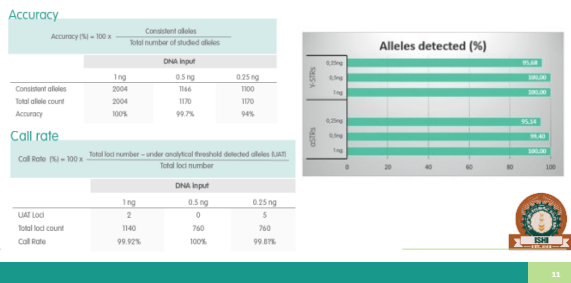


ForenSeq® MainstAY Kit Validation studies
Call rate



10

ForenSeq® MainstAY Kit Validation studies



11

ForenSeq® mtDNA CR Kit Validation studies – preliminary results

Call rate: 99.69%
Concordance: 100%

Input	Reference	Total Read Count	Stranded Depth	Percent A		Percent C		Percent G		Percent T		Percent Del	Percent Ref
				Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp		
2000	A	10390	8700	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2015	C	7724	8807	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
182817	C	7717	4038	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
182817	C	8780	6038	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
181102	T	8961	6427	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Input	Reference	Total Read Count	Stranded Depth	Percent A		Percent C		Percent G		Percent T		Percent Del	Percent Ref
				Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp		
2000	A	14700	10100	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
2015	C	1874	1874	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	19162	8888	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	19762	7580	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
181102	T	17162	1001	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	

Input	Reference	Total Read Count	Stranded Depth	Percent A		Percent C		Percent G		Percent T		Percent Del	Percent Ref
				Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp		
2000	A	16537	7618	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
2015	C	278	2674	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	12770	8611	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	16640	7602	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
181102	T	8454	6048	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	

Input	Reference	Total Read Count	Stranded Depth	Percent A		Percent C		Percent G		Percent T		Percent Del	Percent Ref
				Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp		
2000	A	18441	10880	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
2015	C	4897	8807	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	18276	9888	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
182817	C	18880	10480	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
181102	T	13000	7488	0.2%	0.2%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	

12

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From CE to MPS



13

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MPS in Missing Persons and History Projects



14

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Mass Grave from the Spanish Civil War (1936)

Case description

- 9 tooth samples proceeding from Spanish Civil War Victims
- 4 buccal swabs from possible relatives
- 3 previously obtained profiles (CE) from possible relatives
- Relationships inferred:
 - 4 grand-father-grandson
 - 1 uncle-nephew
 - 1 father-son

Procedure

- Cleaning and decontamination
- O/N incubation in lysis / decalcification buffer
- DNA Extraction with PrepFiler™ BTA (ThermoFischer)
- Quantification with Qualex® Pro II (Qiagen)
- Library preparation with ForenSeq® MainStAY kit (Verogen)



15

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Mass Grave from the Spanish Civil War (1936)

DVI simulation - performed with FAMILIAS v 3.2.g

Based on aSTRs						
Family ID	Relative ID	Unidentif. person	Prior	Posterior	LR	Kinship
Family 1	MD23-30	MD23-30	0,11111	0,999823	4,96E+04	GF-GS
Family 2	MD23-31	MD23-41	0,11111	0,8984	8,59E+01	GF-GS
Family 3	MD23-33	MD23-45	0,11111	0,983047	5,80E+01	U-N
Family 4	MD23-34	MD23-37	0,11111	0,984179	1,99E+02	GF-GS
Family 5	MD23-36	MD23-43	0,11111	0,999999	1,43E+08	F-S

LR calculations based on YHRD (dataset Y23)

Based on YSTRs				
Family ID	Relative ID	Unidentif. person	LR	Kinship
Family 1	MD23-30	MD23-39	1,37E+04	GF-GS
Family 2	MD23-31	MD23-41	3,40E+01	GF-GS
Family 3	MD23-33	MD23-45	6,43E+03	U-N



19

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Identifying remains from the XVIII Century

Case description

- Long bones from 3 different individuals found in a crypt
- 1 dorsal vertebra and a fibula fragment from an indubated individual
- Is any of the three remains found in the crypt a brother of the indubated one?



Procedure

- Cleaning and decontamination
- O/N incubation in lysis / decalcification buffer
- DNA Extraction with PrepFiler™ BTA (ThermoFischer)
- Quantification with Quasiplex® Pro (Qiagen)
- Library preparation with ForenSeq® MantisA kit (Verogen)



20

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Identifying remains from the XVIII Century a-STRs

Sample ID	MD23-17	MD23-18	MD23-19	MD23-22
(DNA) nr./id. di	0,99	0,19	0,84	0,03

	MD23-17	MD23-18	MD23-19	MD23-22
Amelogenin1	X,Y	X,Y	X,Y	X,Y
D16S1656	16:3-17	16:1-3	14:11-3	16:17-3
TPOX	9:1	8-9	8-9	9-11
D2S441	10-13	10	11-14	11-14
D21S1338	19	16-19	17	19-24
D18S1368	15-17	15-17	14-16	14-18
D4S2608	9-11	9-11	10	9-9
FGA	20-24	24	22-27	21-27
D5S818	11	10-11	11-12	9-12
CSF1PO	10-13	11-13	10-11	10
D6S1043	11-12	11-12	11	11-14
D7S820	8-9	9-12	10	10
D8S1179	13-15	14-15	11-13	13-14
D9S1132	12-14	13-14	12	12
D10S1248	14	13-14	16-17	13-17
Th01	6-8	7-9	9-3	9-9-3
vWA	17-18	17-18	15-17	16-16
D12S891	18-20	19-23	19-23	18-19
D13S317	11-12	12	11	9-11
PentaE	2-7	—	10-11	—
D16S839	12-13	12-7	11-13	11-13
D17S1201	12	11-12	12	11-12
D19S11	15-16	13-15	17	13-17
D18S433	14-15	14	14-15	14
D20S442	13-14	14-16	13-15	13-16
D21S11	20-30	20-30	28-31,2	28-31,2
PentaD	11-14	—	12-14	—
D22S1045	15	—	11-16	—



21

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Identifying remains from the XVIII Century Y-STRs



	MD23-17	MD23-18	MD23-19	MD23-22
DYS393	12	12	13	13
DYS662	12	12	12	12
DYS470	17	17	17	17
DYS616	18	18	18	18
DYS622	10	—	10	—
DYS481	23	23	22	22
DYF387N	35-38	35-38	36-38	36-38
DYS19	14	—	14	—
DYS391	10	10	10	10
DYF385	23	—	23	—
DYS437	15	15	14	14
DYS439	11	11	12	12
DYS399	29	—	29	—
DYS398	13	13	13	13
DYS438	12	12	12	12
DYS412	38	38	38	—
DYS390	25	—	24	—
DYS443	10	10	10	10
DYS633	12	12	12	12
Y-GATA-H4	12	12	12	12
DYS385a-b	11-13	11-7	11-14	11-7
DYS460	10	—	10	—
DYS449	14	14	13	13
DYS392	13	—	13	—
DYS448	19	—	17	—



22

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Bone samples studied with ForenSeq® Signature Prep Kit

DNA: 0.008ng/μL (DI=10)

DNA: 0.017ng/μL (DI=3)

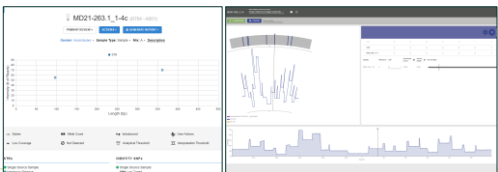


23

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Bone samples studied with ForenSeq® mtDNA CR Kit

- Bone samples proceeding from a mass grave from the Spanish Civil War
- No STRs or SNPs amplified with ForenSeq DNA Signature Prep
- Whole mt DNA CR sequenced with ForenSeq mtDNA Control Region



24

24

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Conclusions

- MPS shows higher sensitivity
- Effective with low DNA input
- Good performance with degraded samples
- More STRs in only one reaction
- Enables to do replicates
- Verogen technology presents two sizes of sequencing kit to support different sample throughput .



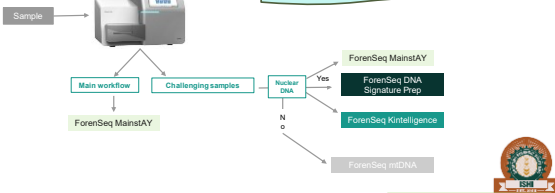
25

25

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Challenge met!

Citogen's MPS workflow



26

26

Thank you!
¡Muchas gracias!

27
